DATA SHEET

AssayMax Human alpha 1-Acid Glycoprotein ELISA Kit

Catalog Number EG5001-1

Introduction

alpha 1-Acid Glycoprotein (AGP) is an acute-phase protein secreted by the liver which under conditions of inflammation increase several-fold in concentration (1). An elevated serum level of acute-phase inflammatory markers is associated with an increased risk of cardiovascular disease Urinary orosomucoid excretion rate predicts cardiovascular mortality in patients with Type II diabetes (2). AGP can be used as a marker for inflammation (3), chronic alcohol drinking (4), chronic kidney disease (5), and asthma (6).

Principal of the Assay

The AssayMax Human AGP ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative competitive sandwich enzyme immunoassay technique that measures human plasma, serum, and urine AGP in less than 3 hours. A polyconal antibody specific for human AGP has been pre-coated onto a 96-well microplate with removable strips. AGP in standards and samples is competed by a biotinylated AGP sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Human AGP Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human AGP.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human AGP Standard:** Human AGP in a buffered protein base (24 μg, lyophilized).
- **Biotinylated AGP:** 1 vial, lyophilized.

- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and Biotinylated AGP at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:1000 into MIX Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:1000 into MIX Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:4 into MIX Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 24 µg of AGP Standard with 1 ml of MIX Diluent to generate a solution of 24 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (24 µg/ml) 1:4 with MIX Diluent to produce 6, 1.5, 0.375, 0.094, and 0.023 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at < -20^{0}C.

Standard Point	Dilution	[AGP] (μg/ml)
P1	Standard (24 μg/ml)	24.000
P2	1 part P1 + 3 parts MIX Diluent	6.000
P3	1 part P2 + 3 parts MIX Diluent	1.500
P4	1 part P3 + 3 parts MIX Diluent	0.375
P5	1 part P4 + 3 parts MIX Diluent	0.094
P6	1 part P5 + 3 parts MIX Diluent	0.023
P7	MIX Diluent	0.000

- **Biotinylated AGP:** Dilute Biotinylated AGP with 4 ml MIX Diluent to produce a working solution. Any remaining solution should be frozen at < -20^{0}C.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 μl of standard or sample per well, and immediately add 25 μl of Biotinylated AGP to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 μl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 μl of Wash Buffer.
- Add 50 μl of Chromogen Substrate per well and incubate for 7 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

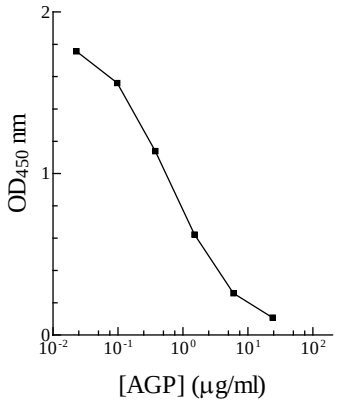
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or semi-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma value by the dilution factor of 1000, serum value by the dilution factor of 1000, and urine value by the dilution factor of 4.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

H. AGP Standard Curve



Precision, Sensitivity and Specificity

- The minimum detectable dose of AGP is typically 20 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.3% and 7.0% respectively.

Cross-Reactivities

Species	% Cross Reactivity
Monkey	< 10 (suggest 1:20 dilution for plasma)
Mouse	None
Rat	< 1
Swine	None
Beagle	None

Recovery

Standard Added Value	0.5 – 5 µg/ml
Recovery %	84-115 %
Average Recovery %	99.5 %

Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:500	97%	95%
1:1000	100%	101%
1:2000	97%	102%

	Average Percentage of Expected Value
Sample Dilution	Urine
1:2	100%
1:4	102%
1:8	101%

References

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(4) Tsutsumi M *et al.* (2001) *Alcohol.* 25(3):181-4
(5) Romao JE Jr. *et al.* (2006) *Am J Nephrol* 26(1):59-66
(6) Van Den Heuvel MM. *et al*. (2000) *Am J Respir Crit Care Med*. 161(6):1972-8

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